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Evaluation of the activity of cyanide-metabolizing sulfurtransferase enzymes in different tissues of turkey (*Meleagris gallopavo*)

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Abstract

This study was undertaken to estimate specific activities of rhodanese and 3-mercaptopyruvate sulfurtransferase (MST) in different tissues of turkey. Enzyme activities were determined in tissue samples from freshly killed adult male turkeys by measurement of thiocyanate amounts produced by the action of enzymes on suitable substrates. Activities of rhodanese and MST in examined tissues of turkey range from 0.023 to 0.448 and 0.01 to 0.191, respectively. The highest activity of the rhodanese was observed in the liver followed by the kidney and then heart, with statistically significant difference between them. Indeed, the activities of MST were significantly higher in hepatic and renal tissues of turkey than in other examined tissues. Although both sulfurtransferases were detected in brain and all studied parts of the digestive tract, the mean values of enzyme activities were far less compared with those of liver and kidney. The results suggest high potential of liver and kidney in sulfurtransferases mediated cyanide detoxification in turkey. However, the demonstration of both enzyme activities in all examined tissues supports the involvement of them in other biochemical processes besides cyanide detoxification which needs to be clarified in detailed studies.

Keywords: Mercaptopyruvate sulfurtransferase, Rhodanese, Tissue distribution, Turkey

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Introduction

Cyanide (CN^-) is a rapid acting cytotoxic agent which inhibits aerobic metabolism by blocking cytochrome oxidase, the terminal oxidase of the mitochondrial electron transport chain (Isom *et al*. 2010). It is one of the most toxic substances, affecting all classes of living cells whether they are plants, animals or microorganisms (Fuller, 1984). Many naturally occurring compounds as well as industrial products contain cyanide (Egekeze and Oehme, 1980). Various species of bacteria, algae, fungi, and higher plants had been identified as natural sources of cyanide (Way, 1984). More than 2000 species of plants are known to contain cyanogenic glycosides (Vennesland *et al.*, 1982) and animals may be exposed to cyanide, released from the glycosides, if their diet contains such materials (Oh *et al.*, 1977). It has been documented that ingestion of cyanogenic glycosides in forage crops can result in the death of grazing animals (Conn, 1978). Deaths of various species of birds from cyanide poisoning have been reported through several routes, including exposure to cyanide salts or ingestion of cyanogenic plants (Wiemeyer *et al.*, 1986). However, various sensitivities to cyanide have been reported in different avian species (Wiemeyer *et al.*, 1986). It has been reported that flesh eating avian species (black vulture, American kestrel and eastern screech-owl) are more sensitive to acute toxicity of sodium cyanide than those species (Japanese quail, domestic chicken and European starling) that fed predominantly on plant material (Wiemeyer *et al.,* 1986).

Sulfuration of cyanide to thiocyanate (SCN^-) is the main in vivo biochemical pathway for cyanide detoxification which is catalysed directly by two sulfurtransferases, thiosulfate: cyanide sulfurtransferase (rhodanese, EC 2.8.1.1) and 3-mercaptopyruvate sulfurtransferase (MST, EC 2.8.1.2) (Nagahara *et al.*, 2003; Isom *et al.*, 2010). These enzymes are discovered over 40 years ago, and have been found to ubiquitously exist in a wide range of species of both prokaryotes and eukaryotes (Isom *et al.*, 2010). Even though these sulfurtransferases metabolize cyanide to a less toxic compound thiocyanate, they have different substrate specificity and tissue distribution pattern (Isom *et al.*, 2010). Moreover, it has been proposed that these enzymes have additional functions; including formation of iron sulfur centers (Cerletti, 1986) and regulation of the cellular sulfane sulfur pool (Isom *et al.*, 2010).

The activities of these enzymes had been studied in the tissues of a wide range of mammalian and avian species (Dudeck *et al.*, 1980; Drawbaugh and Marrs, 1987; Aminlari and Gilanpour, 1991; Aminlari *et al.*, 1994, 2007; Al-qarawi *et al.*, 2001; Agboola *et al.*, 2006; Baghshani and Aminlari, 2009; Shahbazkia *et al.*, 2009), but to our knowledge, no data has yet been reported about their tissue distribution in turkey. The present study was undertaken to determine the distribution of these enzymes in different tissues of turkey.

Materials and methods

Tissue samples (liver, kidney, heart, lung, brain, esophagus, intestine, proventriculus, gizzard, and breast muscle) from freshly killed adult male turkeys (*Meleagris gallopavo*) were obtained from a local slaughterhouse. Samples of each tissue were cleaned free of extraneous material, washed a few times with physiological saline, and then stored at −70°C until analysis. All chemicals utilized were of analytical grade and were supplied by Sigma (St Lewis, Mo, USA) or Merck (Darmstadt, Germany).

Tissue samples were rapidly thawed and homogenized in 10 volumes of ice cold 0.025 M sodium phosphate, pH 7.2. The suspensions were centrifuged at 4°C for 15 min at 4,000×g, and supernatants were used for enzyme assays. Rhodanese was assayed based on the work of Sorbo (1953) as described previously by Aminlari and Shahbazi (1994), in which its activity is efficiently followed by the

production of thiocyanate from thiosulfate and cyanide. 3-mercaptopyruvate sulfurtransferase was assayed based on the work of Taniguichi and Kimura (1974) in which its activity is followed by the production of thiocyanate from 3-mercaptopyruvate (as the sulfur donor) and cyanide (as the sulfur acceptor). Concentration of thiocyanate was determined using ferric nitrate reagent (Sorbo, 1953) and the absorbance of reaction product was detected at 460 nm. The standard curve was prepared with sodium thiocyanate and the units of enzyme activities were defined as micromoles of thiocyanate formed per minute under the assay conditions. Protein concentration was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as standard. Enzyme activities are expressed as specific activity (units per milligram protein).

All results were analyzed using one-way analysis of variance followed by *Bonferroni's* multiple comparisons test. The level of significance was set at $p<0.05$. All calculations were performed using SPSS/PC software.

Results

The activities of the two measured enzymes are presented in table 1 as mean ± SEM.

Table 1. Specific activities (U/mg protein) of rhodanese and mercaptopyruvate sulfurtransferase in different tissues of turkey (n=7). Mean±SEM in each column with no common superscript differ significantly (*p*<0.05)

Tissue	Rhodanese	Mercaptopyruvate sulfurtransferase
Liver	0.448 ± 0.043^{a}	$0.191\pm.011^{a}$
Kidney	$0.323\pm.046^{b}$	0.182 ± 0.013^{a}
Lung	$0.086\pm0.005^{c,d}$	$0.042\pm0.003^{b,c}$
Heart	0.173 ± 0.009^{d}	$0.050\pm.006^{c}$
Brain	0.064 ± 0.012^{c}	$0.024\pm0.005^{b,c}$
Esophagus	0.023 ± 0.003^{c}	$0.016\pm0.002^{b,c}$
Proventriculus	0.036 ± 0.008^{c}	$0.020\pm0.006^{b,c}$
Gizzard	0.027 ± 0.003^{c}	0.010 ± 0.002^{b}
Duodenum	0.054 ± 0.008^{c}	$0.029\pm0.006^{b,c}$
Muscle	$0.107\pm0.009^{c,d}$	0.045 ± 0.008^{c}

The present study results reveal the presence of both enzymes activities in studied tissues, although in different quantities. Mean specific activities of rhodanese and MST in examined tissues of turkey range from 0.023 to 0.448 and 0.01 to 0.191, respectively. The highest specific activity of the rhodanese was observed in the liver followed by the kidney and then heart, with statistically significant difference between them. Based on the present study results, the specific activities of MST were significantly higher in hepatic and renal tissues of turkey than in other examined tissues. However, unlike rhodanese activity, the activities of MST were not significantly different between liver and kidney. The lowest mean activities of both measured enzymes were observed in esophagus and gizzard.

Discussion

Studies on the enzyme profiles of various organs of animal species are of particular importance, as such information can help localize certain biochemical processes that are unique to a tissue and characterize function of the organ. In addition, such information might also provide the basis of a biochemical diagnosis of some disease states in tissues. The distribution of enzymes is markedly different between different organs and animal species (Bailey *et al.*, 1999). The pattern of distribution of rhodanese and MST in different animals appears to be highly species and tissue specific (Aminlari *et al.*, 2007). Indeed, the activity of these enzymes in a particular tissue may reflect the ability of that tissue to detoxify cyanide (Al-Qarawi *et al.*, 2001).

The role of rhodanese in cyanide detoxification has been known for many years

(Sorbo, 1953) and is supported by the high concentration of this enzyme in some mammalian tissues and organs (e.g., the liver) exposed to cyanide (Sylvester and Sander, 1990; Aminlari and Gilanpour, 1991). The highest mean specific activity of rhodanese in the liver of turkey is reminiscent of previously reported data in some mammalian and avian species (Dudeck *et al.*, 1980; Drawbaugh and Marrs, 1987; Aminlari and Gilanpour, 1991; Al-Qarawi *et al.*, 2001; Aminlari and Vaseghi, 2006; Baghshani and Aminlari, 2009) and may be attributed to the major role of this organ in cyanide detoxification process (Aminlari and Gilanpour, 1991; Al-Qarawi *et al.*, 2001; Agboola *et al.*, 2006). It is noteworthy, however, that in some mammalian species other organs such as adrenal glands (Himwich and Saunders, 1948) or some parts of digestive tract (Aminlari *et al.*, 1989; Shahbazkia *et al.*, 2009) have been shown to contain higher activities of rhodanese as compared to hepatic tissue. Moreover, the highest specific activity of this enzyme was found to be in the kidney in some avian species like ostrich (Eskandarzade *et al.*, 2012), Japanese quail (Baghshani and Aminlari, 2009), pigeon (Drawbaugh and Marrs, 1987; Al-Qarawi *et al.*, 2001; Agboola *et al.*, 2006), and domestic fowl (Oh *et al.*, 1977). High rhodanese activity in kidneys might facilitate cyanide detoxification and elimination as SCN (Sylvester and Sander, 1990).

High amounts of rhodanese activity were also found in heart of turkey, although significantly in lower levels than liver and kidney. High activity of rhodanese in turkey heart is consistent with the previously documented findings in ostrich (Eskandarzade *et al.*, 2012), partridge (Baghshani and Aminlari, 2009), chicken (Oh *et al.*, 1977; Aminlari and Shahbazi, 1994) and pigeon (Agbola *et al.*, 2006; Baghshani and Aminlari, 2009). In view of accumulating evidence that covalent modification (by phosphorylation/ dephosphorylation) of rhodanese is involved in the regulation of mitochondrial energy generation (Ogata and Volini, 1990), its high activity in the heart might be attributed to high energy requirements of this tissue which is achieved mainly aerobically. The phosphorylated rhodanese can activate NADH dehydrogenase and an iron-sulfur protein of the respiratory chain (Ogata and Volini, 1990). Considerably high rhodanese activity was also found in turkey muscle that is similar to some extent with previous findings in partridge, and pigeon (Baghshani and Aminlari, 2009), but different from those reported in ostrich (Eskandarzade *et al.*, 2012). Considering the total mass of skeletal muscle, it can be suggested that muscle makes substantial contribution in cyanide biotransformation in the body (Devlin *et al.*, 1989). In the present study, relatively high levels of rhodanese were also found in the lung, which can serve also as another cyanide detoxification site (Sylvester and Sander, 1990). However, despite the present findings, relatively very low activities of rhodanese were reported in lung of Japanese quil, partridge, and pigeaon (Baghshani and Aminlari, 2009) and ostrich (Oh *et al.*, 1977; Agboola *et al.*, 2006; Eskandarzade *et al.*, 2012).

Present findings show apparently that although both sulfurtransferases are detectable in brain and all studied parts of the digestive tract, the mean values are far less compared with those of liver and kidney. The mean tissue specific activities of MST obtained in the present study are lower than that of rhodanese which is in agreement with those reported for some other species (Aminlari *et al.*, 1988; Al-qarawi *et al.*, 2001; Agboola *et al.*, 2006). Moreover, according to the reported findings in some mammalian and avian species (Aminlari *et al.*, 1989; Al-qarawi *et al.*, 2001; Agboola *et al.*, 2006), the present results show that liver and kidney are the richest sources of MST in turkey. However, in the poultry chicken and duck the highest activity level of MST was observed in the gizzard (Agboola *et al.*, 2006). The low levels of rhodanese and MST in turkey brain is in accordance with previously published reports in some other avian species (Eskandarzade *et al.*, 2012).

The distribution pattern of both studied enzymes in the digestive tract of turkey has some notable differences with previously published works in other avian species (Oh *et al.*, 1977; Aminlari and Shahbazi, 1994; Baghshani and Aminlari, 2009; Eskandarzade *et al.*, 2012). For example, according to Oh *et al.,* (1977) and Aminlari and Shahbazi (1994) the chicken proventriculus contained higher activity of rhodanese as compared to other studied parts of digestive tract. Indeed, similar findings regarding high enzymatic activity of rhodanese in proventriculus has been reported in some other avian species (Baghshani and Aminlari, 2009). On the other hand, in ostrich the highest values of rhodanese specific activity (after liver and kidney) have been reported in duodenum and the lowest values were detected in the proventriculus (Eskandarzade *et al.*, 2012). Controversial pattern of sulfurtransferase activities in tissues of various animal species may be due to physiological specificities, dietary constituents, differences in the mean age and weight of sampled animals, and differences in procedures used for enzymes assays or other unknown factors.

Based on the present study results, the richest sources of both measured sulfurtransferases in turkeys are hepatic and renal tissues which are likely to play a substantial role in cyanide detoxification. However, the demonstration of both rhodanese and MST activities in all examined tissues of turkey, suggests that these enzymes are also involved in other biochemical processes besides cyanide detoxification which needs to be clarified precisely in detailed biochemical studies.

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ارزیابی میزان فعالیت آنزیم های سولفورترانسفراز متابولیزه کننده سیانید در بافت های مختلف بوقلمون (*Meleagris Gallopavo*)

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چکیده

مطالعه حاضر به منظور تعیین فعالیت مخصوص رودنیز و ۳-مرکاپتوپیروات سولفور ترانسفراز (MST) در بافتهای مختلف بوقلمون انجام گرفت. در نمونه های بافتی اخذ شده از بوقلمون های تازه کشتار شده، فعالیت های آنزیمی با اندازه گیری میزان تیوسیانات تولید شده طی عملکرد آنزیم بر سوبستراهای مناسب تعیین گردید. محدوده فعالیت آنزیم های رودنیز و MST در بافتهای مورد مطالعه بوقلمون به ترتیب ۰.۴۴۸-۰.۰۲۳ و ۰.۱۹۱-۰.۰۱ بود. بیشترین میزان فعالیت رودنیز در کبد و پس از آن در کلیه و سپس در قلب مشاهده شد که اختلاف معنی دار بین آنها نیز وجود داشت. به علاوه، فعالیت MST در بافتهای کبد و کلیه بطور معنی داری بیشتر از سایر بافتهای مورد مطالعه بود. با وجود این که هردو آنزیم سولفور ترانسفراز در مغز و تمام قسمتهای بررسی شده دستگاه گوارش وجود داشتند میانگین مقادیرفعالیت آنزیمی این بافتها در مقایسه با کبد و کلیه بسیار پایین بود. نتایج حاضر نشان دهنده ی پتانسیل بالای کبد و کلیه در سم زدایی سیانید به وسیله ی سولفور ترانسفراز ها می باشد. با این وجود، مشاهده فعالیت هر دو آنزیم در تمام بافتهای مورد بررسی می تواند دلالت بر نقش داشتن این آنزیم ها در سایر فرایندهای بیوشیمیایی علاوه بر سم زدایی سیانید باشد که شناسایی آنها مستلزم تحقیقات بیشتر می باشد.

واژگان کلیدی: مرکاپتو پیروات سولفور ترانسفراز، رودنیز، توزیع بافتی، بوقلمون